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Spatial variations in salinity stress across a coastal landscape using vegetation indices derived from hyperspectral imagery

#### **ABSTRACT**

Chlorophyll fluorescence and landscapelevel reflectance imagery were used to evaluate

spatial variations in stress in Myrica cerifera and Iva frutescens during a severe drought and compared to an extremely wet year. Measurements of relative water content and the water band index (WBI970) indicated that the water stress did not vary across the island. In contrast, there were significant differences in tissue chlorides across sites for both species. Using the physiological reflectance index (PRI), we were able to detect salinity stress across the landscape. For M. cerifera, PRI did not differ between wet and dry years, while for I. frutescens, there were differences in PRI during the 2 years, possibly related to flooding during the wet year. There was a positive relationship between PRI and DF=F0m for M. cerifera (r2 = 0.79) and I. frutescens (r2 = 0.72). The normalized difference vegetation index (NDVI), the chlorophyll index (CI), and WBI970 were higher during the wet summer for M. cerifera, but varied little across the island. CI and WBI970 were higher during 2004 for I. frutescens, while there were no differences in NDVI during the 2 years. PRI was not significantly related to NDVI, suggesting that the indices are spatially independent. These results suggest that PRI may be used for early identification of salt stress that may lead to changes in plant distributions at the landscape level, as a result of rising sea level. Comparsions between the two species indicate that variations in PRI and other indices may be species specific.

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# Spatial variations in salinity stress across a coastal landscape using vegetation indices derived from hyperspectral imagery

Julie C. Naumann · Donald R. Young · John E. Anderson

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**Abstract** Chlorophyll fluorescence and landscapelevel reflectance imagery were used to evaluate spatial variations in stress in Myrica cerifera and Iva frutescens during a severe drought and compared to an extremely wet year. Measurements of relative water content and the water band index (WBI<sub>970</sub>) indicated that the water stress did not vary across the island. In contrast, there were significant differences in tissue chlorides across sites for both species. Using the physiological reflectance index (PRI), we were able to detect salinity stress across the landscape. For M. cerifera, PRI did not differ between wet and dry years, while for I. frutescens, there were differences in PRI during the 2 years, possibly related to flooding during the wet year. There was a positive relationship between PRI and  $\Delta F/F'_{\rm m}$  for M. cerifera ( $r^2 = 0.79$ ) and I. frutescens  $(r^2 = 0.72)$ . The normalized difference vegetation index (NDVI), the chlorophyll index (CI), and WBI<sub>970</sub> were higher during the wet summer for M. cerifera, but varied little across the island. CI and WBI<sub>970</sub> were higher during 2004 for I. frutescens, while there were no differences in NDVI during the 2 years. PRI was not significantly related to NDVI, suggesting that the indices are spatially independent. These results suggest that PRI may be used for early identification of salt stress that may lead to changes in plant distributions at the landscape level, as a result of rising sea level. Comparsions between the two species indicate that variations in PRI and other indices may be species specific.

**Keywords** PRI · NDVI · WBI<sub>970</sub> · Chlorophyll fluorescence · Salinity · Hyperspectral reflectance

### Introduction

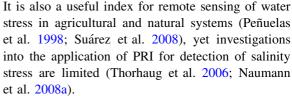
Salinity is considered to be the primary environmental factor influencing community patterns in coastal ecosystems (Oosting and Billings 1942; Ehrenfeld 1990; Stalter and Odum 1993). Drought, high irradiance, and high temperatures are among many other factors that limit plant growth in these environments (Ehrenfeld 1990). These physical forces create distinct zones of vegetation across the coastal landscape relative to distance from the ocean. Environmental boundaries are not as discrete as the zonation of coastal plant communities; yet there is a relationship between changes in species composition and gradient for abiotic stressors (Crawford 1989). Young et al. (1994) demonstrated that spatial and seasonal

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J. E. Anderson Fluorescence Spectroscopy Lab, US Army ERDC, 7701 Telegraph Road, 22315 Alexandria, VA, USA variations in groundwater salinity and soil chlorides are partly responsible for the spatial distribution of woody vegetation on barrier islands. Shifts in plant distributions due to salinity in coastal areas are likely to occur with the predicted effects of climate change, most notably sea-level rise and increased storm intensity and frequency (Gregory and Oerlemans 1998; Zhang et al. 2000).

Much effort has been devoted to identifying stress in plants before visible signs are observed. Changes in visible reflectance, shifts in the reflectance curve red edge, and differences in various indices have correlated strongly with plant stress (Carter 1993; Carter and Young 1993; Blackburn 2007). In recent years, attention has been focused on measurements of chlorophyll fluorescence as a means of early stress detection. However, research into light-adapted measurements of fluorescence is imperative, as darkadaptation is not currently feasible at scales beyond the leaf level. Chlorophyll fluorescence measurements are ideal because they are non-destructive and linked to physiological functioning of plants (Zarco-Tejada et al. 2000). One drawback to fluorescence is that most instruments are not capable of making measurements from a distance because of the weak signal (less than 2% of total reflected visible light). Thus, finding the fluorescence signal in reflectance data has been a focus of more recent research efforts and would enable rapid large-scale detection of plant physiological status (Zarco-Tejada et al. 2003; Evain et al. 2004; Dobrowski et al. 2005).

All environmental stresses that lower the photosynthetic rate of a plant will increase the need for energy dissipation of excess, absorbed light (Demmig-Adams and Adams 1992). One mechanism for the dissipation of excess energy involves changes in xanthophyll cycle pigments. Under excess light, violaxanthin is converted into zeaxanthin. Increases in zeaxanthin levels are correlated to increases in energy dissipation, which can be measured by chlorophyll fluorescence (Demmig-Adams Adams 1996). The increased levels of zeaxanthin can also be monitored by changes in reflectance at 531 nm using the physiological reflectance index (PRI). PRI has been successfully used to indicate physiological changes from both acute (Evain et al. 2004; Dobrowski et al. 2005; Naumann et al. 2008a) and chronic stress (Filella and Peñuelas 1999; Asner et al. 2004; Filella et al. 2004; Peñuelas et al. 2004).



Applicability of PRI at the landscape (defined here as across a scene) and larger scales is complicated due to the heterogeneous composition of the landscape and other errors (Peñuelas et al. 1997). Recently, researchers have demonstrated the success of using airborne imagery for calculating PRI in various systems (Asner et al. 2004; Fuentes et al. 2006; Suárez et al. 2008; Naumann et al. 2008b). The objective of our study was to identify spatial variations in plant stress in the dominant woody vegetation on a Virginia barrier island, using a combination of field measurements and hyperspectral airborne reflectance. Specific goals were to (1) identify areas of stress using field measurements of chlorophyll fluorescence, (2) determine the cause of stress, (3) link field measurements to hyperspectral imagery, and (4) use hyperspectral imagery and indices to identify stress across the landscape.

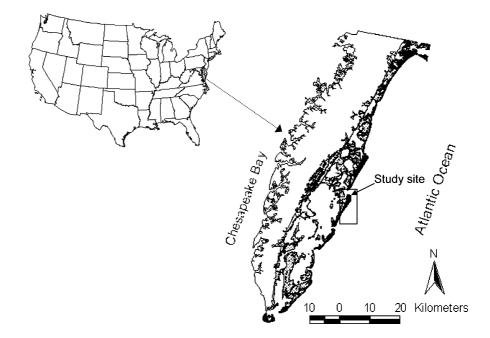
#### Materials and methods

Study sites

The field study was conducted on September 13, 2007 on the North end of Hog Island (37°40' N; 75°40′ W), a barrier island located on the Eastern Shore of Virginia (Fig. 1). The Oceanside, northern end of the island, has been accreting approximately 5 m/year for 140 years (Hayden et al. 1991), resulting in a parallel series of dunes and swales. We conducted our study across the northern end of the island, focusing on two shrubs: Myrica cerifera L. (Myricaceae), an evergreen, nitrogen-fixing, salt sensitive shrub, which dominates the mesic swales and Iva frutescens L. (Asteraceae), a salt-succulent shrub most common along the edge of salt marshes (Ehrenfeld 1990; Young et al. 1994). Five M. cerifera and two I. frutescens sites were used in our study with five sampling locations at each site (Fig. 2). Study sites were chosen based on exposure to salinity. The M. cerifera Oceanside site is  $\sim 200$  m from the Atlantic Ocean and the most exposed to salt



**Fig. 1** Location map for study site at Hog Island, Virginia



spray. The Backside site is the leeward, protected side of the same thicket. The Young site is  $\sim 30$  m from the Backside site and is ocean facing. The Dune site is located in the middle of the island adjacent to a dune and samples were collected from the ocean facing side. The Mid-island site is in the middle of the island adjacent to a freshwater pond, and is generally the most freshwater flooded site. Samples taken from the Mid-island site faced away from the ocean. The two *I. frutescens* sites are located on the bayside of the island. The Bayside site is located at the edge of the saltwater marsh, while the Path site occurs at a higher elevation along a dry path and is adjacent to a hypersaline saltpan (Fig. 2).

## Field measurements

Monthly variations in precipitation during 2004 and 2007 were obtained from a meteorological station on Hog Island (Krovetz et al. 2007). Light-adapted chlorophyll fluorescence was measured at each site (n=50) using a pulse amplitude modulated leaf fluorometer (PAM-2000, Walz, Effeltrich, Germany). The relationship between maximal fluorescence in a light-adapted leaf after a saturating pulse of light  $(F_{\rm m}')$  and steady-state fluorescence prior to any saturating pulse  $(F_{\rm s})$  was used to estimate the effective quantum yield of photosystem II:

$$\Delta F/F'_{\rm m} = \left[F'_{\rm m} - F_{\rm s}\right]/F'_{\rm m}$$

Five leaves from each sampling location were clipped at the stem and kept at 100% humidity. Relative water content was measured as

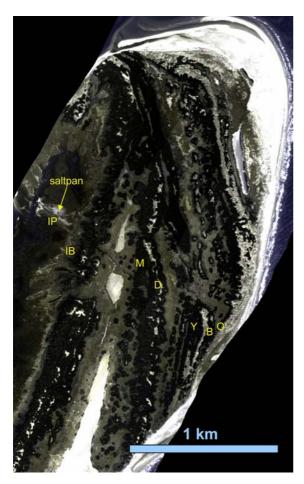
$$RWC(\%) = (FW - DW)/(SFW - DW) * 100$$

where FW is fresh weight, DW is dry weight, and SFW is saturated fresh weight of the leaves after rehydrating samples for 24 h (Turner 1981). Tissue chlorides were quantified at each sampling location (n = 5). Leaf samples were oven-dried at 80°C for 72 h and then ground in a fine mesh mill. For each sample, 0.5 g of material was placed in a tube with 40 ml of deionized water. Samples were placed in a boiling water bath for 2 h, cooled, and then filtered into 100-ml volumetric flasks. To each sample, 2 ml of 5 M NaNO<sub>3</sub> was added as an ionic equalizer, and then samples were brought to volume with deionized water (Young et al. 1994). Chloride levels were determined using a chloride electrode (model 9617b, Orion, Boston, MA).

# Airborne image acquisition

The airborne hyperspectral mission was flown concurrent with the field measurements at Hog Island on September 13, 2007. On the day of the flight, air





**Fig. 2** SpectIR hyperspectral image of sites at Hog Island, Virginia. The following letters denote *M. cerifera* thickets used in the study: O, Oceanside; B, Backside; Y, Young; D, Dune; M, Mid-island. The *I. frutescens* sites in the study are: IB, Bayside; IP, Path

temperature was 29°C with relative humidity of 45% and 2076 μmol m<sup>-2</sup> s<sup>-1</sup> PPFD at solar noon. Hyperspectral data (3 nm resolution) were provided by the SpectIR using the ProSpecTIR VIS hyperspectral imaging spectrometer (SpectIR Corp.). Hyperspectral imagery covering 450–2,450 nm was collected under cloud-free conditions at 1,700 m (AGL), providing a dataset representing 2 m/pixel on the ground and a final spectral cube 356 bands deep. These data products were post-processed to correct for geometric and radiometric (e.g., bi-directional) effects. Ground reflectance radiometry was used to calibrate the data based on target endmembers collected in-scene with the ASD FieldSpec Pro Full Range reflectance radiometer (Analytical Spectral Devices, Inc.,

Boulder, CO). This effectively placed the scene into reflectance units and helped to negate any atmospheric effects. Calibration was performed using the empirical line calibration method within ENVI (RSI, Inc.). Points were selected from each sampling location based on GPS measurements and the corresponding spectra were extracted. The extraction of pixels with 2 m resolution enabled the calculation of indices without any shadowing effects. Numerous reflectance indices were calculated to elucidate spatial variation across the island. Only the relevant indices are included in this aarticle and are listed in Table 1.

The summer of 2007 was very dry. In order to understand the relationships in spectral indices across the island, we also acquired imagery of Hog Island during an unusually wet year. The airborne hyperspectral mission was flown on August 24, 2004 using the Portable Hyperspectral Imager for Low-Light Spectroscopy (PHILLS) (Davis et al. 2002). On the day of the flight, air temperature was 29°C with relative humidity of 71% and 2018 μmol m<sup>-2</sup> s<sup>-1</sup> PPFD at solar noon. Hyperspectral imagery covering 384–1,000 nm was collected at a spatial resolution of 3 m/pixel. These data products were post-processed to correct for geometric and radiometric (e.g., bidirectional) effects. Ground reflectance radiometry was also used to calibrate the data based on target endmembers collected in-scene with a handheld spectroradiometer (Analytical Spectral Devices, Inc., Boulder, CO). Transformation of the PHILLS data was accomplished using the SpectIR data as a reference to normalize the bands. Extraction of pixels and subsequent calculation of indices at specific wavelengths from the 2004 PHILLS data were normalized to the reference 2007 SpectIr data using a histogram matching procedure following Jensen (2005). The equation for this procedure is

$$DN_{new} = (DN_t - MEAN_t/STD_t) STD_r + MEAN_r$$

where  $DN_{new}$  denotes the new spectral band(s) (PHILLS),  $DN_t$  denotes the digital number values for the image to be transformed (PHILLS band), MEAN $_t$  denotes the statistical mean of the image to be transformed (PHILLS band), STD $_t$  denotes the standard deviation of the image to be transformed (PHILLS band), STD $_r$  denotes the standard deviation of the reference image (SpectIR band), and MEAN $_r$  denotes the statistical mean of the image to be



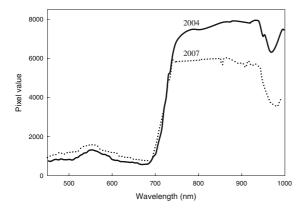
**Table 1** Vegetation indices used in our statistical analyses

Reflectance index	Formula	Reference
Physiological reflectance index (PRI)	$(R_{531} - R_{570})/(R_{531} + R_{570})$	(Gamon et al. 1992)
Normalized difference vegetation index (NDVI)	$(R_{801} - R_{670})/(R_{801} + R_{670})$	(Daughtry et al. 2000)
Chlorophyll index (CI)	$(R_{750} - R_{705})/(R_{750} + R_{705})$	(Gitelson and Merzlyak 1996)
Water band index (WBI <sub>970</sub> )	$R_{970}/R_{900}$	(Peñuelas et al. 1993)

transformed (SpectIR band). Given the close acquisition times, spectral, spatial, radiometric and ground features for each airborne mission, this effectively normalized each image set for comparative stress calculations based upon reflectance signatures. Representative reflectance spectra of a Mid-island site obtained from the flights are shown in Fig. 3.

#### Statistical analyses

Analysis of variance (ANOVA) was used to test for variations in sites of the each species for the following measurements: chlorophyll fluorescence, RWC, and tissue chlorides (Zar 1999). Significant differences among sites were identified with Tukey tests ( $\alpha=0.05$ ). Two-way ANOVA was used to test for significant interactions between site and year for reflectance indices. In cases where a significant interaction occurred, one-way ANOVAs were used to test for variations among sites within a year and to test for variations between years at a specific site. Variations in reflectance indices were related to variations in chlorophyll fluorescence, RWC, and tissue chlorides using linear regressions.

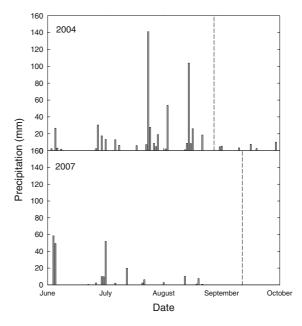


**Fig. 3** Representative reflectance spectra from the 2004 PHILLS and 2007 ProSpecTIR VIS hyperspectral flights

#### Results

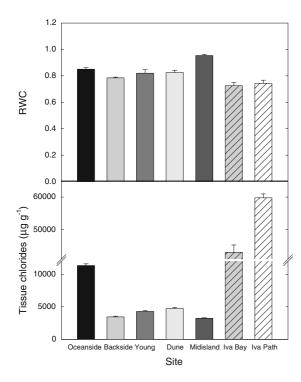
Precipitation for June through September of 2004 was 52% above the 30-year average (388 mm). In comparison, 2007 was unusually dry and characterized by a persistent drought; precipitation for June through September was 39% below the 30-year average (Fig. 4). In 2004, the longest period without rainfall was 11 days in June, whereas in 2007 only two rain events >5 mm occurred after July 11 and no precipitation occurred after mid-August (Fig. 4).

In 2007, relative water content for *M. cerifera* averaged  $96 \pm 1\%$  at the Mid-island site and was significantly higher compared to other island sites (F = 16.76, P < 0.001). Average RWC ranged from  $85 \pm 1\%$  to  $78 \pm 0\%$  at the other *M. cerifera* sites (Fig. 5). RWC did not significantly differ between



**Fig. 4** Monthly variations in precipitation as measured from a meteorological station on Hog Island between June 1 and September 30 for 2004 and 2007. The *dotted lines* represent the flight dates during each year

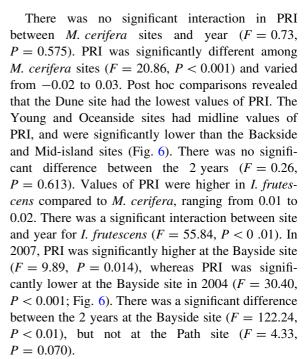




**Fig. 5** Variations in relative water content (RWC) (a) and tissue chlorides (b) across the island for both *M. cerifera* and *I. frutescens* sites during 2007. Values represent means  $\pm$  1 standard error

I. frutescens sites (F = 0.26, P = 0.621) and averaged  $73 \pm 2\%$  (Fig. 5). Total chlorides present in leaves were higher for the salt succulent plant, I. frutescens compared to M. cerifera (Fig. 5). Among M. cerifera sites, the Oceanside thicket had the highest chlorides and was significantly different from all other sites (F = 261.72, P < 0.001). The Young and Dune sites were similar in tissue chloride concentrations, as were the Mid-island and Backside sites (Fig. 5). Tissue chlorides were significantly lower for I. frutescens at the Bayside site compared to the Path site (F = 42.43, P < 0.001; Fig. 5).

 $\Delta F/F_{\rm m}'$  significantly differed across the island in M. cerifera thickets ( $F=19.29,\ P<0.001$ ). The Dune site had the lowest  $\Delta F/F_{\rm m}'$  values (0.48  $\pm$  0.03) and was significantly lower than all other sites (Fig. 6). Highest  $\Delta F/F_{\rm m}'$  values were found at the Backside (0.70  $\pm$  0.03) and Mid-island sites (0.68  $\pm$  0.02). There was a significant difference in  $\Delta F/F_{\rm m}'$  of I. frutescens between the Bayside (0.73  $\pm$  0.01) and Path sites (0.70  $\pm$  0.02;  $F=9.89,\ P=0.014$ ; Fig. 6).



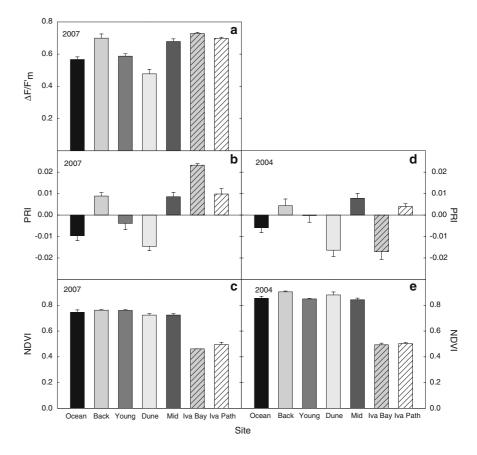
There was a significant interaction in normalized difference vegetation index (NDVI) between M. cerifera sites and year (F = 2.77, P = 0.040; Fig. 6). There were significant differences among sites with the highest values of NDVI at the Backside site (F = 5.46, P = 0.002), and there was a significant difference between years (F = 227.24, P < 0.001). In 2007, NDVI values ranged from 0.73 to 0.77 across sites, while values were much higher in 2004, ranging from 0.82 to 0.94. NDVI values at the I. frutescens sites were much lower compared to M. cerifera and ranged from 0.46 to 0.57. There was no significant interaction between year and site for I. frutescens (F = 0.94, P = 0.346; Fig. 6), and there were no significant differences between years (F = 2.75,P = 0.117) and sites (F = 3.14,P = 0.095).

PRI was positively related to  $\Delta F/F_{\rm m}'$  ( $r^2=0.79$ , P<0.001; Fig. 7) at *M. cerifera* sites. PRI decreased from 0.014 to -0.019, while  $\Delta F/F_{\rm m}'$  decreased from 0.78 to 0.40 with highest  $\Delta F/F_{\rm m}'$  recorded at the Backside site. For *I. frutescens*, PRI was positively related to  $\Delta F/F_{\rm m}'$  ( $r^2=0.72$ , P=0.002; Fig. 7).

PRI was not related to RWC in *M. cerifera*  $(r^2 = 0.09, P = 0.135; \text{ Fig. 8})$  or *I. frutescens*  $(r^2 = 0.00, P = 0.994; \text{ Fig. 8})$ . There were no significant relationships between  $\Delta F/F'_{\text{m}}$  and RWC in *M. cerifera* or *I. frutescens*  $(r^2 = 0.09, P = 0.151;$ 



Fig. 6 Variations in  $\Delta F/F'_m$  (a), PRI (b), and NDVI (c) across the island during 2007 for both *M. cerifera* and *I. frutescens* sites. Variations in PRI (d) and NDVI (e) during 2004 are also presented. Values represent means  $\pm$  1 standard error



 $r^2 = 0.02$ , P = 0.701, respectively). There was a weak but significant negative relationship between PRI and tissue chlorides in M. cerifera ( $r^2 = 0.27$ , P = 0.008; Fig. 9). The Oceanside site had extremely high tissue chlorides compared to the other sites, which is likely influenced by salt spray on the leaves. When this site was removed from the regression, a much stronger relationship between PRI and tissue chlorides emerged ( $r^2 = 0.81$ , P < 0.001; Fig. 9). There was also a negative relationship between PRI and tissue chlorides for I. frutescens ( $r^2 = 0.71$ , P = 0.002; Fig. 9). A similar pattern was seen between  $\Delta F/F'_{\rm m}$  and tissue chlorides. With all M. cerifera sites included, there significant relationship  $(r^2 = 0.11,$ was P = 0.100), but once the Oceanside site was removed, a strong relationship was seen ( $r^2 = 0.72$ , P < 0.001). For *I. frutescens*, a significant relationship was also seen between  $\Delta F/F'_{\rm m}$  and tissue chlorides ( $r^2 = 0.45$ , P = 0.033).

In 2007, PRI was not significantly related to NDVI at the *M. cerifera* sites  $(r^2 = 0.04, P = 0.637;$ 

Fig. 10), suggesting that PRI is not tracking changes in NDVI and the indices are spatially independent. For *I. frutescens*, PRI was related to NDVI, but this relationship was not considered significant at  $\alpha = 0.05$  level ( $r^2 = 0.38$ , P = 0.059; Fig. 10). Similar trends were seen in 2004 for PRI and NDVI among *M. cerifera* sites ( $r^2 = 0.00$ , P = 0.707) and *I. frutescens* sites ( $r^2 = 0.00$ , P = 0.938; Fig. 10).

There were no significant relationships between chlorophyll index (CI) and  $\Delta F/F_{\rm m}'$  among M. cerifera sites ( $r^2=0.05$ , P=0.292). There was a significant interaction in CI between site and year for M. cerifera (F=4.31, P=0.005). CI was significantly higher in 2004 (F=92.80, P<0.001). There were no differences among sites in 2004 (F=0.75, P=0.568). In 2007, CI was significantly lower at the Mid-island site (F=8.97, P<0.001). For I. frutescens, CI exhibited a relationship with  $\Delta F/F_{\rm m}'$  but was not significant ( $r^2=0.35$ , P=0.071). There was no interaction between site and year for I. frutescens (F=0.39, P=0.539). CI was significantly higher in 2004 (F=64.18,



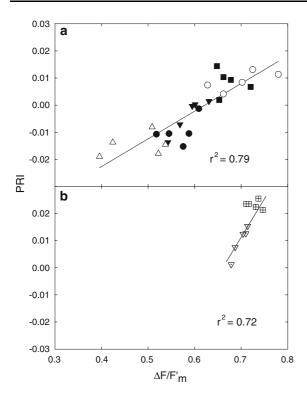
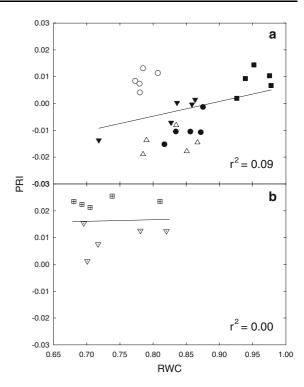


Fig. 7 Relationship between PRI and  $\Delta F/F'_m$  for M. cerifera sites (a) and I. frutescens sites (b), where lacktriangle, Oceanside;  $\bigcirc$ , Backside;  $\P$ , Young,  $\triangle$ , Dune; and  $\blacksquare$ , Mid-island M. cerifera sites, and  $\boxminus$ , Bayside and  $\P$ , Path I. frutescens sites

P < 0.001), but there were no differences between sites (F = 0.45, P = 0.514). Water band index (WBI<sub>970</sub>) was not related to  $\Delta F/F_{\rm m}'$  at the M. cerifera sites or the *I. frutescens* sites ( $r^2 = 0.08$ , P = 0.183;  $r^2 = 0.00$ , P = 0.906, respectively) in 2007. Among M. cerifera sites, there was no interaction between site and year (F = 1.95, P = 0.121). WBI<sub>970</sub> was significantly higher during 2004 (F = 41.32, P < 0.001) and the Mid-island site was significantly higher than other sites (F = 5.43, P < 0.001). There was a marginally significant interaction between year and site for *I. frutescens* (F = 4.26, P = 0.056). WBI<sub>970</sub> was higher during 2004 (F = 277.55, P < 0.001) and was higher at the Bayside (F = 35.64, P = < 0.001). For M. cerifera, WBI<sub>970</sub> was significantly related to RWC in 2007 ( $r^2 = 0.69$ , P < 0.001; Fig. 11), but there was no relationship between WBI970 and RWC for I. frutescens  $(r^2 = 0.00, P = 0.910; Fig. 11).$ 

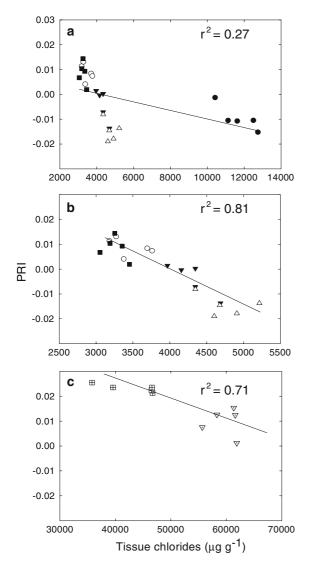


**Fig. 8** Relationship between PRI and RWC during 2007 for *M. cerifera* site (**a**) and *I. frutescens* (**b**). Symbols are defined in Fig. 5

#### Discussion

The results of our field study show a strong link between  $\Delta F/F'_{\rm m}$  and PRI for both M. cerifera and I. frutescens on a Virginia barrier island. These findings are similar to studies focused on water stress, which have shown positive relationships between PRI and fluorescence (Peñuelas et al. 1998; Winkel et al. 2002). During the same 2007 field season, Naumann et al. (2008b) showed that M. cerifera experienced a drought response, as seen in decreases in stomatal conductance, photosynthesis, and RWC relative to earlier in the season. Chlorophyll fluorescence did not respond to drought, but rather to differences in salinity. In this study the cause of stress is attributed to variations in salinity rather than drought based on tissue chlorides and RWC, as well as a comparison in reflectance data from the dry summer of 2007 with a relatively wet summer in 2004. Salinity affects plant water status and produces a suite of effects similar to those caused by drought in newly developed transpiring leaves (Munns 2002).





**Fig. 9** Relationship between PRI and tissue chlorides during 2007 for all *M. cerifera* sites (**a**), for *M. cerifera* sites with the Oceanside site removed (**b**), and for *I. frutescens* sites (**c**). Symbols are defined in Fig. 5

Previous research has shown declines in chlorophyll fluorescence and PRI to be indicators of salinity stress in *Myrica cerifera* prior to visible signs of stress in both laboratory (Naumann et al. 2007) and field experiments (Naumann et al. 2008b).

PRI has been correlated to plant water status under drought conditions (Suárez et al. 2008). In our study, spatial variations in PRI and  $\Delta F/F_{\rm m}'$  were not linked to variations in water content during the summer drought of 2007, but did relate to tissue chlorides across the island. This is further supported by the

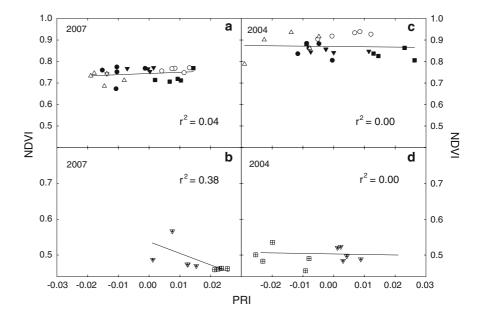
similar pattern of spatial variation in PRI for M. cerifera during the unusually wet summer of 2004. Across the coastal landscape, M. cerifera is restricted to well-defined mesic swales due to sensitivity to moisture stress (Young 1992). However, distance from the shoreline and distance to the water table affect soil moisture content at a given landscape position, and therefore every thicket differs considerably in soil water availability (Shao et al. 1995). Thus, in an extremely wet summer, we would expect to see less variation in PRI across the landscape if the spatial variations seen in 2007 were due to microsite differences in drought stress. NDVI values were higher in M. cerifera thickets during the wet summer of 2004 relative to 2007, in agreement with values of annual shoot growth across the island from these years (Donald R. Young unpublished data).

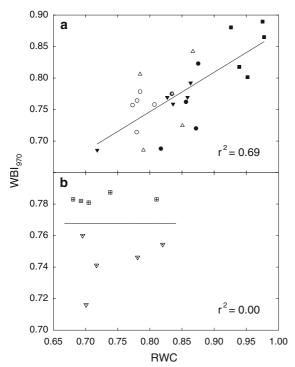
CI and WBI<sub>970</sub> were also higher in 2004, consistent with expectations for a wet year. In 2007, CI was lower at the Mid-island site, but aside from this, there were no differences across sites, suggesting that differences in chlorophyll content are not responsible for changes in  $\Delta F/F'_{\rm m}$  or PRI across the island. Although there were no significant differences in WBI<sub>970</sub> across the island, it did exhibit a good relationship with RWC for M. cerifera, suggesting that WBI<sub>970</sub> is a good index for monitoring the water status in this species. There was no relationship between WBI970 and RWC for I. frutescens, but WBI<sub>970</sub> was higher at the Bayside site. Because this site is frequently flooded, these plants are likely to have access to more water than the Path site, resulting in a higher WBI<sub>970</sub>, while microsite differences in salinity may influence the RWC of each plant (Hacker and Bertness 1995).

Differences in PRI,  $\Delta F/F_{\rm m}'$ , and tissue chlorides from the Oceanside and Backside of the same thicket, which are separated by a distance of no more than 50 m, are of interest. The Backside site did not differ significantly from the Mid-island site ( $\sim 900$  m inland) in terms of PRI, fluorescence, and tissue chlorides. Aspect and distance from the ocean are very important in determining the effect of salinity on plants and creating spatial variation in salinity stress across the landscape (Ehrenfeld 1990; Young et al. 1995). Sea spray appears to be a cause of salinity at least among some of the sites. For example, the Backside site, while only 250 m away from the shoreline, is the protected, leeside of the thicket and thus does not receive as much sea spray as sites that



Fig. 10 Relationship between PRI and NDVI during 2007 for *M. cerifera* sites (a) and *I. frutescens* sites (b), and during 2004 for *M. cerifera* sites (c) and *I. frutescens* sites (d). Symbols are defined in Fig. 5





**Fig. 11** Relationship between WBI<sub>970</sub> and RWC during 2007 for *M. cerifera* (**a**) and *I. frutescens* (**b**). Symbols are defined in Fig. 5

are ocean facing. The Oceanside site had extremely high values for tissue chlorides relative to other sites; yet the Dune site had the lowest values of PRI and  $\Delta F/F_{\rm m}'$ . The high values of chlorides at the Ocean-side site may be influenced not only by those in the tissues, but also by chlorides from sea spray impact to the surface of the leaf, which is likely to be pronounced in an extremely dry season.

Sea spray is difficult to quantify both in space and time, especially on a remote island where access is limited (Ehrenfeld 1990). Based on our chloride data and the lack of coastal storms during the study period, the accumulations of tissue chlorides in this study are most likely due to sea spray. Storm overwash can also lead to increase in salinity levels, but previous work showed that groundwater salinity remained relatively low (less than 3 g l<sup>-1</sup> on the bayside and lower inland) in M. cerifera thickets across the island even following a major northeastern storm (Young et al. 1991). The Backside site had lower tissue chlorides compared to the Young site, which faces the ocean but is further inland, again suggesting influence from sea spray. The Dune site, while further inland, is a very dry site and may be influenced by other factors, such as depth to the water table, which could cause the effects of salinity to be greater. More study is needed to assess the exact cause of stress at this site. The Mid-island site, which is the most protected, appeared to be the least stressed site from field measurements and reflectance data.

For the halophyte *I. frutescens*, tissue chlorides were much higher and RWC values were



considerably lower compared to the salt and moisture sensitive plant M. cerifera. Measurements of  $\Delta F/F'_{\rm m}$ and PRI were also higher in *I. frutescens* during 2007. We did not expect to see such low values of PRI in 2004. The higher 2007 PRI values could be due to low salinity effects from increased freshwater input during the wet summer; it could be a function of flooding at the site or a combination of both. Thorhaug et al. (2006) showed that decreased salinity reduced PRI in halophytic seagrass. However, Iva frutescens generally only occurs at elevations where the roots are not subject to prolonged water table flooding (Bertness et al. 1992). The Bayside site is at the edge of the marsh at a lower elevation than the Path site. Mean elevation of the water table at the Bayside site in 2004 was 0.5 m higher than mean elevation in drier years (Brinson 2007). Despite the inconsistency at the Bayside site in 2004, differences in PRI during 2007 can be explained by variations in salinity across the island.

In 2007, PRI was lower at the Path site and tissue chlorides were much higher compared to the Bayside site. This is due to periodic flooding during extreme high tides at the Path site and subsequent evaporation resulting in higher levels of soil salinity (Hayden et al. 1995). Approximately 15 m from the Path site lays a hypersaline saltpan (Fig. 1). In comparison, the Bayside site lies in a tidal area and is flooded daily. Thus, soil salinity levels are not as likely to build up due to the constant input of water (Hayden et al. 1995). NDVI values did not differ between years for I. frutescens and were much lower compared to M. cerifera. No significant relationship between PRI and NDVI in either species suggests that factors such as LAI and leaf angle were not affecting the PRI signal and that PRI can be used at the landscape level in these species.

Hyperspectral measurements over homogenous ecosystems are lacking (Inoue et al. 2008). Barrier islands are model systems for remote sensing because of the homogeneous community composition. Our study demonstrates the usefulness of PRI for remote detection of salinity stress in *M. cerifera* thickets. These thickets are ideal because they form dense, monotypic canopies with very high LAI, reducing the confounding effects of canopy structure and heterogeneous composition for applying PRI at the landscape scale (i.e., across the island; Brantley and Young 2007). For instance, comparison between

2 years of hyperspectral data is likely not confounded by soil background due to the high LAI of *M. cerifera*. For *I. Frutescens*, there may be some interference of soil background, particularly during a drought year, where the canopy may not be as full. Caution should be taken in assessing stress in *I. frutescens* using PRI. Knowledge of the system is important for correct interpretation of PRI values and the cause of stress. Regardless, PRI successfully identified areas of stress across the landscape.

#### Conclusion

Spatial variations in stress were detected on the barrier island using chlorophyll fluorescence, which were related to variations in tissue chlorides for both M. cerifera and I. frutescens. Salinity appeared to be a factor responsible for patterns of stress across the landscape, and was detectable using PRI from airborne hyperspectral imagery. Variations in PRI remained constant during a wet and dry year for M. cerifera, while NDVI, CI, and WBI970 were higher during the wet summer, but varied little across the island. Thus, PRI was the most useful index for stress detection in M. cerifera. For I. frutescens, PRI was related to chloride concentrations during the dry year, but a different pattern in PRI emerged during a wet year, suggesting that this index is useful in detecting stress, but the cause may not always be obvious. These findings, especially for *M. cerifera*, have implications for monitoring the effects of climate change in coastal systems. Our results suggest that PRI may be used for early identification of salt stress that may lead to changes in plant distributions at the landscape level as a result of rising sea level and increased storm intensity. In order to effectively use PRI to detect the effects of climate change, further investigation of the sources of salinity, the impact of drought, and associated interactions on island vegetation is needed.

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